L Number	Hits	Search Text	DB	Time stamp
1	121836	cross-link$	USPAT; US-PGPUB; EPO; DERWENT	2003/06/18 12:50
2	37495	allophycocyanin or aps or xl-aps	USPAT; US-PGPUB; EPO; DERWENT	2003/06/18 12:50
3	2008	sodium adj perchlorate	USPAT; US-PGPUB; EPO; DERWENT	2003/06/18 12:51
4	4892	cross-link$ and (allophycocyanin or aps or xl-aps)	USPAT; US-PGPUB; EPO; DERWENT	2003/06/18 12:51
5	21	(sodium adj perchlorate) and (cross-link$ and (allophycocyanin or aps or xl-aps))	USPAT; US-PGPUB; EPO; DERWENT	2003/06/18 12:51

L Number	Hits	Search Text	DB	Time stamp
1	2008	sodium adj perchlorate	USPAT; US-PGPUB; EPO; DERWENT	2003/06/18 12:34
2	1541	allophycocyanin	USPAT; US-PGPUB; EPO; DERWENT	2003/06/18 12:34
3	4	(sodium adj perchlorate) and allophycocyanin	USPAT; US-PGPUB; EPO; DERWENT	2003/06/18 12:34

L17 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2

TI Crosslinking of **allophycocyanin**

AB Crosslinking of trimeric **allophycocyanin**, (.alpha..beta.)3, with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide leads to the formation of .alpha.-.beta. as the only major intersubunit crosslinked product. This result is consistent with an alternating arrangement of .alpha. and .beta. subunits in the disk-shaped **allophycocyanin** trimer. After purifn. by gel filtration in 8M urea at pH 3.0, and renaturation, the crosslinked species reassembles to form an (.alpha.-.beta.)3 trimer. The (.alpha.-.beta.)3 trimer has spectroscopic properties very similar to those of untreated **allophycocyanin** trimer. Whereas **allophycocyanin** dissocs. to monomers at very low protein concn., or in the presence of **chaotropic** ions such as thiocyanate, or upon exposure to maleic anhydride, the crosslinked (.alpha.-.beta.)3 trimer does not dissoc. under any of these conditions.

SO Physiologie Vegetale (1985), 23(5), 777-87
CODEN: PHYVAP; ISSN: 0031-9368

AU Ong, Linda J.; Glazer, Alexander N.

ANSWER 3 OF 5 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3

TI Stability of **allophycocyanin**'s quaternary structure

AB The dissocn. of **allophycocyanin** trimers to monomers was examd. under a variety of conditions. For alkyl ureas and alcs., the dissocn. increased as the straight-chain alkyls increased in length. The effect of branching chains was smaller. Tetrapropylammonium chloride was an effective agent for trimer dissocn. when compared to ureas and alcs. with similar or longer alkyl chains. These hydrocarbons apparently have an affinity for nonpolar regions in the contact areas between monomers in a trimeric structure. A comparison among several inorg. salts demonstrated that the **chaotropic** salts (NaSCN > NaClO4 .mchgt. NaNO3 > NaBr) fostered increased trimer dissocn., whereas nonchaotropes (KF, (NH4)2SO4, K-phosphate, and NaCl) produced no measurable amts. of monomer. **Allophycocyanin** dissolved in D2O was much more stable against dissocn. than when dissolved in H2O. The above observations are consistent with hydrophobic forces being the dominant source of trimer stabilization. The equil. const. for the dissocn. of trimers to monomers was .apprx.6 .times. 10-16 mol2 L-2. Calcns. were made of the apparent total no. of amino acids (40) in the 2 contact regions on each monomer. An absorption change analogous but not necessarily identical to a conversion of **allophycocyanin** II to III was noted when (NH4)2SO4 was present. When **allophycocyanin**'s nonexchangeable hydrogens were placed by deuteriums, it more readily dissocd. to monomers.

SO Archives of Biochemistry and Biophysics (1983), 223(1), 24-32
CODEN: ABBIA4; ISSN: 0003-9861

AU MacColl, Robert

ANSWER 7 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 7

TI Methods of identifying nuclear receptor ligands using fluorescence resonance energy transfer (FRET)

AB This invention provides methods of identifying novel agonists and antagonists of nuclear receptors utilizing the agonist-dependent interaction of such receptors with co-activators, in which this interaction is detected by fluorescence resonance energy transfer (FRET). Specifically, the invention involves anal. of agonist-dependent binding of CREB-binding protein (CBP) with peroxisome proliferator-activated receptors (PPARs). In the absence of agonist, binding between the nuclear receptor and CBP does not occur, but if the agonist is present, such binding occurs and can be detected by FRET using a fluorescent-labeled nuclear receptor and fluorescent-labeled CBP. The binding of antagonist ligands to nuclear receptors prevents recruitment of co-activator CBP, and thus, antagonists can be identified by virtue of their ability to prevent or disrupt the agonist-induced interaction of nuclear receptors and CBP. The invention provides a nuclear receptor, or ligand binding domain thereof, and co-activators, or binding portions thereof, labeled with europium (EuIII) or terbium(TbIII) cryptates as donor fluorescent reagents, or XL665 (a **cross-linked allophycocyanin**).

SO PCT Int. Appl., 60 pp.
CODEN: PIXXD2

IN Cummings, Richard T.; Hermes, Jeffrey D.; Moller, David E.; Zhou, Gaochao

L18 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 8

TI Development of a CD28/CD86 (B7-2) binding assay for high throughput screening by homogeneous time-resolved fluorescence

AB CD28 has been demonstrated to provide the major costimulatory signal for CD4-pos. T cells. Ligation with its natural ligands CD80 (B7-1) and CD86 (B7-2) leads to signals during activation that are required for the prodn. of interleukin-2, and this process has been implicated in the regulation of T-cell anergy and programmed cell death. This article describes the assay development, assay validation, and primary screening for small mol. antagonists of this interaction, which could be potential drug candidates. The assay uses homogeneous time-resolved fluorescence based on energy transfer from excited europium ions to **cross-linked allophycocyanin**, which then subsequently emits a fluorescent signal. An "indirect" approach was taken, whereby the **cross-linked allophycocyanin** (XL665) is covalently linked to an antihuman antibody that binds to a human Ig domain fused to CD28. The CD86 that is expressed as a fusion protein with a rat Ig domain is bound to biotinylated sheep antirat antibody, which is complexed with streptavidin-europium cryptate. This "cassette" format facilitates the development of related assays using CTLA-4 in place of CD28 and/or CD80 in place of CD86, allowing easy detn. of the selectivity of active compds. When the CD28 and CD86 are in close proximity (i.e., bound), there is a specific time-resolved emission at 665 nm that is largely absent in either unbound partner. Expts. to optimize the reagent concns., incubation time, solvent effects and quench effects by colored compds. are discussed, as are the results from robustness testing and data from primary screening.

SO Journal of Biomolecular Screening (1998), 3(2), 91-99
CODEN: JBISF3; ISSN: 1087-0571

AU Mellor, Geoffrey W.; Burden, M. Neil; Preaudat, Marc; Joseph, Yvonne; Cooksley, Susan B.; Ellis, Jonathan H.; Banks, Martyn N.

L18 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 5

TI High throughput screening using HTRF

AB A review with 12 refs., on the principle of HTRF (homogeneous time-resolved fluorescence) using europium cryptate and XL665 (a **cross-linked allophycocyanin**), and its application in high throughput screening of novel drugs. Assays for the detn. of proteases and cytokines are introduced. Practical hints for the establishment of assay system using HTRF, and robot systems for HTRF are also discussed.

SO Kagaku to Seibutsu (2000), 38(7), 481-487
CODEN: KASEAA; ISSN: 0453-073X

AU Takemoto, Hiroshi